



UNITED STATES PATENT AND TRADEMARK OFFICE

I, Michael Amos WHITTAKER BA, MCIL, MITI,
translator to RWS Group Ltd, of Europa House, Marsham Way, Gerrards Cross,
Buckinghamshire, England declare;

1. That I am a citizen of the United Kingdom of Great Britain and Northern Ireland.
2. That I am well acquainted with the German and English languages.
3. That the attached is, to the best of my knowledge and belief, a true translation into the English language of the accompanying copy of the specification filed with the application for a patent in Germany on December 19, 2002 under the number 102 59 821.5 and the official certificate attached thereto.
4. That I believe that all statements made herein of my own knowledge are true and that all statements made on information and belief are true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application in the United States of America or any patent issuing thereon.

A handwritten signature in black ink, appearing to read "M. Whittaker".

For and on behalf of RWS Group Ltd

The 3rd day of December 2008

PCT/DE03/04137

FEDERAL REPUBLIC OF GERMANY

[rubber stamp]



Priority Certificate for the filing of a Patent Application

File Reference: 102 59 821.5

Filing date: 19 December 2002

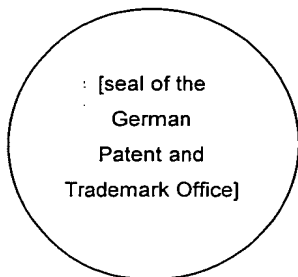
Applicant/Proprietor: Siemens Aktiengesellschaft, 80333 Munich/DE

Title: Bio-Chip

IPC: G 01 N, C 12 M

The attached documents are a correct and accurate reproduction of the original submission for this application.

Munich, 13 January 2004
German Patent and Trademark Office
The President
pp



[signature]
Wallner

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH
RULE 17.1(a) OR (b)

Description

Biochip

The invention relates to a biochip in particular a DNA chip, in accordance with the preamble of the patent claim.

Biochips or DNA chips comprise a flat carrier, on one side of which at least one spot array, that is to say a gridlike arrangement of analysis positions, is present. The spots contain probe or catcher molecules, for example oligonucleotides, immobilized on the carrier surface. Target molecules, for example DNA fragments, contained in an analyte solution applied to a spot couple to the catcher molecules. The conversion of such coupling or binding events into detectable signals is effected by means of optical, piezoelectric, electrochemical, calorimetric or impedance-spectroscopic methods.

In the case of an impedance-spectroscopically readable DNA chip disclosed in DE 196 10 115 C2, an interdigital electrode arrangement is present on a sensor area, catcher molecules being immobilized on the electrodes and the areas arranged between the electrodes. The coupling of target molecules to the catcher molecules leads, e.g. on account of charge changes, to a change in the alternating electric field generated by the electrodes or generally to a change in an electrical property in the vicinity of the electrodes, e.g. the impedance. A measurement of an impedance change can be carried out by means of a, for example, two-pole interdigital electrode arrangement in which the electrodes are formed from a plurality of partial electrodes. What is problematic with this manner of detecting binding events is that the dimensions of the electrode structures differ by orders of magnitude from molecular dimensions. With a technical outlay that is still tenable, it is possible to produce electrodes whose width and spacing,

taken together, have a value L of approximately 2-20 μm and a height of approximately 0.1 to 0.5 μm .

The impedance-spectroscopically detectable range of the electric field of such an electrode arrangement extends approximately 1 to 5 L ($= 2$ to 100 μm) beyond the carrier surface or the planar plane spanned by the electrode arrangement. By contrast, a catcher molecule having 100 base pairs, for example, has a length of only approximately 30 nm. The influence of binding events in a monomolecular layer of catcher molecules that is immobilized on the sensor surface or the electrodes on the electric field is correspondingly low, particularly when only few binding processes take place. "Nanoscaled interdigitated electrode arrays for biochemical sensors", P. van Gerwen et al, Sensors and Actuators B 49, 1998, 72 - 80, proposes, for solving the problem discussed, approximating the dimensions of electrode structures to the dimensions of DNA target molecules, electrode structures with partial electrodes being sought whose widths and mutual spacings lie approximately in the range of 250 to 500 nm. However, such dimensions are associated with an increased production outlay.

It is an object of the invention to propose an impedance-spectroscopically readable DNA chip with improved sensitivity which can be produced cost-effectively.

The object is achieved according to the invention by means of the features of patent claim 1. In the case of the invention, the electrode arrangement is at least partially embedded in a hydrophilic reaction layer which is permeable to target molecules and in which immobilized catcher molecules are distributed three-dimensionally. In this case, the reaction layer is dimensioned such that it is pervaded by the predominant part of the electric field generated by the electrode arrangement or by the impedance-spectroscopic

detection range thereof. One advantage of such a biochip is firstly that a significantly larger number of catcher molecules can be arranged within the reaction layer than in a unimolecular layer on the carrier surface and on the surfaces of the electrodes. Added to this, however, is the fact that the dimensioning of the reaction layer is adapted to the space pervaded by the electric field or by the field lines thereof, so that a large number or a high concentration of catcher molecules is present with an approximately homogeneous distribution within the detection range mentioned. The consequence is a much greater influencing of the electric field or of the impedance-spectroscopic detection range of the electrode arrangement. A DNA chip configured in this way has a correspondingly greater measurement sensitivity, or sensitivity.

In a preferred refinement, the reaction layer has a thickness lying approximately in the range of 1 - 5 L. This ensures that, on the one hand, a region of the electric field with a relatively high field line density is utilized for detecting binding events and, on the other hand, the thickness of the reaction layer is not so large that it impedes the indiffusion of target molecules and reactants. Given electrode widths in the region of approximately 1 μm and the same spacings, the thickness of the reaction layer is between 2 and 100 μm .

With a reaction layer that is thermally stable up to approximately 95°C, a DNA chip of the type under discussion can be employed for PCR reactions. In this case, thermally stable is intended to mean that the reaction layer, even at the temperature mentioned, is configured in such a way that it does not resolve, that it fixedly retains catcher molecules, that reactions between target and catcher molecules can take place unimpeded in it, and that it also essentially maintains its other properties. In a further preferred refinement, the reaction layer contains polymers with coupling groups to which

catcher molecules are covalently bound. This reliably ensures that binding pairs comprising target and catcher molecules are retained in the reaction layer in the event of rinsing operations during the analysis procedure. A particularly suitable reaction layer comprises a hydrogel. Hydrogels form an aqueous milieu in a mechanically stable form which permits a substance exchange with a predominantly aqueous analyte. Acrylamide-based radical-crosslinkable hydrogels with maleic anhydride and/or glycidyl (meth)acrylate as coupling groups have proved to be particularly suitable.

In a further preferred embodiment, the flat carrier of the DNA chip comprises a silicon layer and an insulating layer connected thereto, the latter carrying the electrode arrangement and the reaction layer on its side remote from the silicon layer. In the case of such an arrangement, it is possible to realize the electrical interconnection of the electrode structure with analog and digital circuits known from Si semiconductor technology.

Further details and advantages of the invention emerge from the description of figures of exemplary embodiments with reference to the drawing in conjunction with the patent claims. In the figures:

- Fig. 1 shows a simplified perspective illustration of a biochip comprising a flat carrier and a spot array,
- Fig. 2 shows a cross section through a spot in accordance with line II-II in fig. 1, in an enlarged detail illustration,
- Fig. 3 shows a detail from an electrode arrangement assigned to a spot,
- Fig. 4 shows an embodiment of a biochip with a 4-pole electrode arrangement in an illustration corresponding to fig. 2, and

Fig. 5 shows the electrode arrangement of the biochip of fig. 4 in an illustration corresponding to fig. 3.

As shown in fig. 1, a biochip 1 comprises a flat carrier 2, on one side of which a spot array 3 is applied. A spot 4 contains immobilized catcher molecules, for example oligonucleotides. If an analyte solution with unknown target molecules is applied to a spot, then the target molecule is coupled to the catcher molecule in the event of corresponding matching in the base sequence. The property change brought about by such a binding event, e.g. changes in the resistivity or the dielectric constant, is detected impedance-spectroscopically by means of an electrode arrangement 5.

A 2-pole electrode arrangement is present in the case of the exemplary embodiment of fig. 2. This arrangement is applied to the flat carrier 2 for example with the aid of a photolithographic method. The electrode arrangement 5 comprises two electrodes 6, 7 designed in the form of an interdigital structure. That is to say that each electrode comprises a plurality of strip-type partial electrodes 6a, 7a which run parallel to one another and in each case extend into the interspace between two partial electrodes of the respective other electrodes. The partial electrodes 6a, 7a are connected to one another by a likewise strip-type connecting conductor 6b, 7b extending transversely with respect to the partial electrodes 6a, 7a. An AC voltage e.g. in the megahertz range is applied to the electrodes 6, 7. The width 8 of the partial electrodes 6a, 7a is approximately 1 μm , their height 9 is approximately 100 to 500 nm. A spacing 10 of likewise approximately 1 μm is present between the partial electrodes 6a, 7a.

The flat carrier 2 comprises a silicon layer 12 and an insulating layer 13 e.g. made of silicon dioxide or silicon nitride that is arranged between said silicon layer and the electrodes 6, 7. The electrical interconnections and components

required for the impedance-spectroscopic measurement of binding events are realized in a conventional manner by means of a corresponding patterning of the silicon layer (not illustrated). A reaction layer 14 made of a hydrogel is applied on the insulating layer 13, which hydrogel is described in more detail further below.

Catcher molecules 15 are embedded and homogeneously distributed in the reaction layer 14 or the hydrogel, said catcher molecules being illustrated symbolically and in overdimensioned fashion in fig. 2. A catcher molecule with 300 bases has approximately a length of 100 nm. Accordingly, a unimolecular layer of catcher molecules in the case of conventional biochips has at most approximately a thickness corresponding to the line 16 in fig. 2. It is readily apparent that such a layer can take up relatively few catcher molecules 15 and, correspondingly, can influence the electric field only to a small extent in the case of binding events. By contrast, in the case of a biochip according to the invention, the reaction region that contains catcher molecules and is pervaded by field lines is substantially extended and offers space for a number of target molecules 15 that is greater by a plurality of powers of ten. If an analyte solution 18 is applied to a spot array 3 configured in such a way or to a spot 4, then the target molecules 19 contained in it, which is likewise illustrated only symbolically and with exaggerated size in fig. 2, find a substantially larger number of possible binding partners in the form of the catcher molecules 15. The reaction layer 14 is preferably dimensioned, or has a thickness, such that the impedance-spectroscopic detection range is practically fully utilized, which is the case given a thickness of the reaction layer of approximately 2 to 100 μm . Consequently, the binding-specific measuring effect of the biochip can be substantially increased given a corresponding concentration of catcher molecules 15 in this region.

The reaction layer 14 is configured such that it provides an aqueous reaction medium. Furthermore, it is configured such that target molecules 19 or else other substances required for a reaction, for example polymerase, can indiffuse into it without its activity being impaired in the process.

As already mentioned above, according to the invention a hydrogel is used as the reaction layer 14. A hydrogel represents an aqueous milieu in a mechanically stable form whilst at the same time ensuring the substance exchange in a predominantly aqueous environment. Through the choice of chemical composition, which relates to the components and the ratio thereof among one another, the properties of the hydrogels such as water content, swelling behavior, mechanical stability etc. can be varied over wide ranges.

A hydrogel that can be produced easily and has a good adhesion both with respect to the electrode arrangement 5 and with respect to the insulating layer 13 is an acrylamide-based radical-crosslinkable hydrogel containing a comonomer enabling a covalent coupling of correspondingly modified catcher molecules via linker groups. The hydrogel comprises, in addition to the monomer precursor of polyacrylamide, a crosslinking agent, at least one radical initiator, at least one comonomer with reactive linker groups and, if appropriate, at least one plasticizer. After layer production and subsequent thermal crosslinking or photocrosslinking, a water-swellaable hydrogel is obtained containing reactive linker groups for the immobilization of catcher molecules. Methylene bisacrylamide and/or dimethylacrylates, for example tetraethylene glycol dimethacrylate, are used as crosslinking agents.

The hydrogel mesh size can be set by varying the concentrations of the crosslinking agent. The comonomer used contains maleic anhydride and/or glycidyl (meth)acrylate. Mono-, di- and/or triethylene glycol is suitable as plasticizer. The starting

substances mentioned are mixed with a polar, water-miscible solvent, preferably with dimethylformamide.

The processing viscosity can be set by varying the proportion of solvent. The adhesion to the flat carrier surface and also to the electrode arrangement 5 can be reinforced by admixture of customary adhesion promoters, for example silane-based adhesion promoters.

Figs. 4 and 5 illustrate an exemplary embodiment with a 4-pole electrode arrangement 20. The electrode arrangement 20 is composed of two current electrodes 22, 23 and two voltage or probe electrodes 24, 25. The current electrodes 22, 23 are arranged and configured in accordance with the electrode arrangement 5 of the exemplary embodiment according to fig. 2. The probe electrodes 24, 25 are likewise strip-type and extend as a meandering double strand through the interspaces present between the partial electrodes 22a and 23a. A high-frequency AC current is applied to the current electrodes 22, 23. A voltmeter 26 is connected to the probe electrodes 24, 25 and can be used to detect an alteration in the alternating electric field on account of binding events.

The measurement can thus be effected independently of the current electrodes, so that e.g. their polarization that increases the electrode impedance cannot affect the measurement. By contrast, in the case of a 2-pole electrode arrangement, the electrode impedance has to be kept low by means of a correspondingly high measurement frequency that is unfavorable in terms of measurement technology, in order to be able to determine the resistance of the analyte solution or of the reaction layer, which resistance is crucial for the measurement.

Patent claims

1. A DNA chip comprising a flat carrier (2) and, arranged thereon, an array (3) of spots (4) containing catcher molecules, each spot (4) being assigned a microelectrode arrangement (5) for detecting binding events between the catcher molecules and target molecules applied by means of an analyte solution, characterized in that the electrode arrangement (5) is at least partially embedded in a hydrophilic reaction layer (14) which is permeable to target molecules and in which immobilized catcher molecules are distributed three-dimensionally.
2. The DNA chip as claimed in claim 1, characterized in that the reaction layer has a thickness laying approximately in the range of 1 to 5 L, where L is the sum of electrode width and electrode spacing.
3. The DNA chip as claimed in claim 2, characterized by a thickness of the reaction layer (14) of 2 to 100 μm .
4. The DNA chip as claimed in one of claims 1 to 3, characterized in that the reaction layer (14) is thermally stable up to approximately 95°C.
5. The DNA chip as claimed in one of claims 1 to 4, characterized in that the reaction layer (14) contains coupling groups for the covalent binding of catcher molecules.
6. The DNA chip as claimed in one of claims 1 to 5, characterized in that the reaction layer (14) is a hydrogel.
7. The DNA chip as claimed in claim 6, characterized by an acrylamide-based radical-crosslinkable hydrogel with maleic anhydride and/or glycidyl (meth)acrylate as coupling groups.

10259821.5
200206756

- 10 -

8. The DNA chip as claimed in one of claims 1 to 7, characterized by an interdigital electrode arrangement (5).

9. The DNA chip as claimed in one of claims 1 to 8, characterized in that the flat carrier (2) comprises a semiconductor layer and an insulating layer (13) connected thereto, the latter carrying the electrode arrangement (5) and the reaction layer (14) on its side remote from the semiconductor layer.

10. The DNA chip as claimed in claim 9, characterized in that the semiconductor layer is a silicon layer (12).

10259821.5
200206756

- 11 -

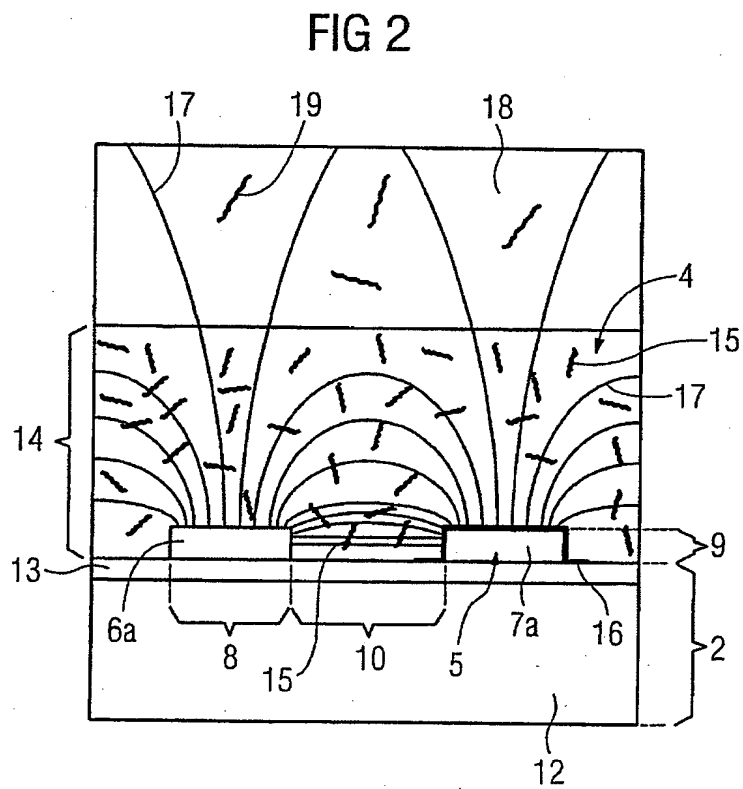
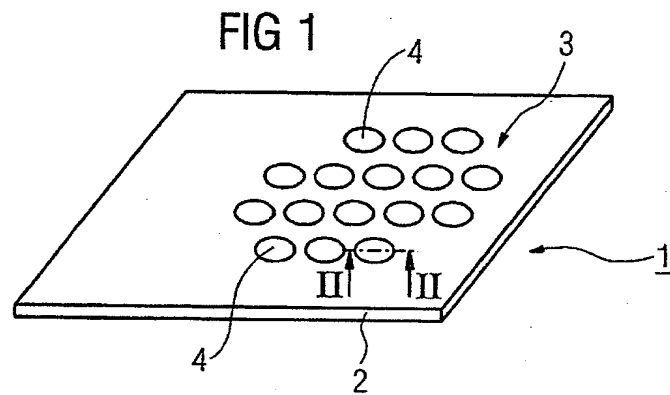
Abstract

Biochip

The invention relates to a biochip comprising a flat carrier and, arranged thereon, an array of spots containing catcher molecules, each spot being assigned a microelectrode arrangement for impedance-spectroscopic detection of binding events between the catcher molecules and target molecules applied by means of an analyte solution. To increase the sensitivity and/or the binding-specific measurement effect of the biochip, the electrode arrangement is at least partially embedded in a hydrophilic reaction layer which contains catcher molecules and is permeable to target molecules.

FIG. 2

1/3



2/3

FIG 3

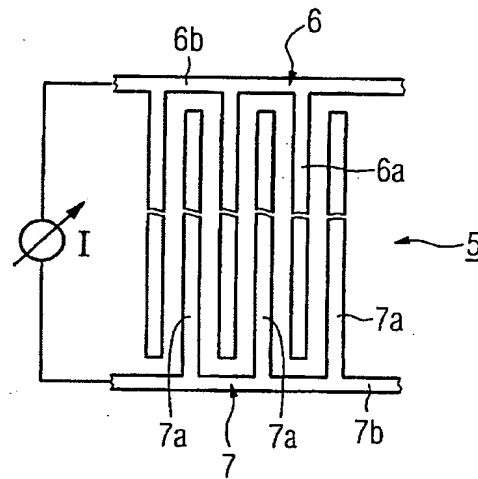


FIG 4

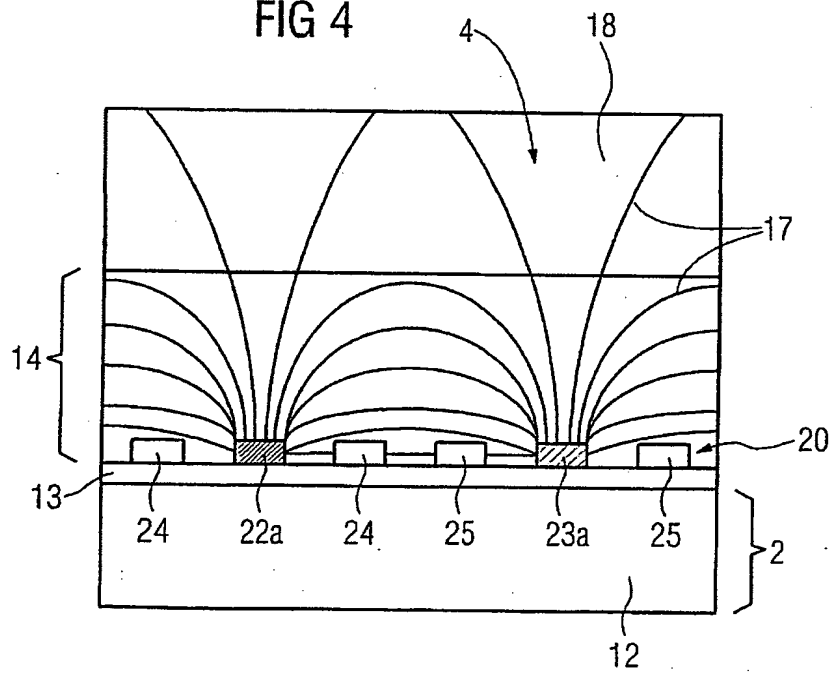


FIG 5

